

Comparative and Cumulative Energetic Costs of Odontocete Responses to Anthropogenic Disturbance

Dawn P. Noren & Marla M. Holt

NOAA NMFS Northwest Fisheries Science Center

2725 Montlake Blvd. East

Seattle, WA 98112

phone: (206) 302-2439 fax: (206) 860-3335 email: dawn.noren@noaa.gov
phone: (206) 860-3261 fax: (206) 860-3335 email: marla.holt@noaa.gov

Robin Dunkin & Terrie M. Williams

Ecology and Evolutionary Biology Dept

University of California, Santa Cruz

100 Shaffer Road

Santa Cruz, CA 95060

phone: (831) 334-0640 fax: (831) 459-3383 email: dunkin@biology.ucsc.edu
phone: (831) 459-5123 fax: (831) 459-3383 email: williams@biology.ucsc.edu

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LONG-TERM GOALS

Cetacean responses to marine anthropogenic activities include changes in acoustic behavior, surface behavior, dive behavior, direction of travel, and behavioral activity states. However, the consequences of these behavioral responses are often difficult to quantify in biological currencies. Previous studies, including our ONR-supported work on the metabolic costs of communicative sound and click production and the metabolic costs of changes in vocal behavior in bottlenose dolphins, have empirically measured the energetic consequences of these behavioral responses that may have acute or chronic impacts. The current investigation involves separate but related studies that will address energetic costs of behavioral responses to anthropogenic disturbance, including acoustic effects, in odontocetes. The first component addresses, in a comparative framework, metabolic costs of sound production and vocal modification across different sound types and odontocete species. The second component addresses cumulative energetic costs of behavioral responses to disturbance. These analyses will provide quantitative information that can be incorporated into models such as the Population Consequence of (Acoustic) Disturbance (PCAD/PCoD) as well as provide input data for environmental assessments/impact statements and other permit processes involving anthropogenic activities that have the potential to impact marine mammals.

OBJECTIVES

This investigation comprises five major objectives, executed over three years. The objectives are: (1) compare the metabolic costs of social sound (e.g., whistle) production with click production in bottlenose dolphins using previously collected empirical data to determine if distinct sound types

confer different energetic costs to the signaler, (2) determine the mass of sound producing muscles in bottlenose dolphins and other representative odontocete species from CT scans, (3) develop a predictive bioenergetic model of the metabolic costs of sound production across several odontocete species from the mass of sound producing muscles (4) conduct myoglobin, acid buffering, and muscle fiber typing analyses to gain insight into physiological performance and potential interspecific differences in energetic demand of sound producing muscle groups, and (5) quantify the cumulative energetic effects of responses to anthropogenic disturbance, in terms of increased energy expenditure, relative to the daily activity budget.

APPROACH

Objective 1: Our previous related work measured the metabolic costs of producing social sounds (whistles and squawks, Noren et al., 2013 and Holt et al., 2015) and clicks (Noren et al., in internal review and MBholt report on award no. N0001412IP20072) in the same two bottlenose dolphins. Direct comparisons of metabolic costs of producing these different sound types are complicated by differences in methodology and energy content of the acoustic signals produced during the separate experiments. In this analysis, Dr. Marla Holt (NOAA NWFSC) compared the metabolic costs of social sounds and clicks, relative to appropriate baseline values, for equal energy sound generation.

Objective 2: Across a variety of species, the relative size of a species' sound-producing muscles are related to the metabolic cost of acoustic signaling (Ophir et al. 2010). Accordingly, we determined the mass of sound-producing muscle in relation to total body mass to predict the metabolic cost of sound production in several odontocete species. Dr. Ted Cranford (SDSU), working in collaboration with Dr. Robin Dunkin (UCSC), analyzed previously collected CT scans from freshly dead cetaceans (code 2) that did not have significant trauma or emaciation to estimate the volume of key muscles associated with sound production (e.g. nasal musculature, key gular muscles, palatopharyngeal muscle). Species included harbor porpoise (*Phocoena phoecena*, n = 3), bottlenose dolphin (*Tursiops truncatus*, n = 2), killer whale (*Orcinus orca*, n = 2), and two beaked whale species (*M. densirostris* = 1, *Z. cavirostris* = 1). Dr. Dunkin converted muscle volume to mass, using the density of muscle, and sound production muscle mass was compared across species.

Objective 3: Dr. Robin Dunkin is using the results from the CT analysis, along with our data on the metabolic cost of sound production in bottlenose dolphins, to develop a model that predicts the cost of sound production across a number of species that vary widely in body size and mass.

Objective 4: Histochemical and biochemical techniques are being used to assess the aerobic and anaerobic capacities of cetacean vocal musculature from several odontocete species (bottlenose dolphin, harbor porpoise, and killer whale). Animals were obtained opportunistically through stranding networks, and individuals were sampled by UCSC and NWFSC PI's and collaborators. Muscles known to play a role in sound production [nasal musculature (NM) around the phonic lips, which includes the posterior externus, anterior externus, posterior internus, and intermedius muscles (Mead 1975), the palatopharyngeal sphincter (PPS), which is involved in pressurizing air within the nasal complex during sound production (Green et al., 1980; Lawrence & Schevill, 1965), and the genioglossus complex (GGC, comprised of the genioglossus, hyoglossus, and styloglossus muscles), which is involved with both sound production and prey consumption (Green et al., 1980; Lawrence & Schevill, 1965)] were sampled and stored frozen at -80°C until analyses were conducted. When available, matched locomotor muscle (*longissimus dorsi*) samples were analyzed for comparison. Dr. Nicole Thometz (UCSC) conducted the analyses to determine myoglobin content ([MB]) and acid

buffering capacity (AB) of muscle samples following the methods of Reynafarje (1963) and Castellini & Somero (1981), respectively. Fiber typing of muscle samples (methods in Dearolf et al. 2000) will be conducted in collaboration with Dr. Jenn Dearolf (Hendrix College). These results will provide additional insight into the energetic cost of sound production across a variety of odontocetes.

Objective 5: Dr. Dawn Noren will estimate the energetic impact of disturbance for several odontocetes species over a range of scenarios. Disturbance scenarios and odontocete responses will be based on species-specific findings from a literature review and will include different sound exposure types (e.g. sonar, vessel noise), levels of exposure (e.g. duration, intensity), and magnitudes of responses (types of behavior and physiologic/energetic effects). Energetic costs of vocal responses (determined in the present investigation), changes in swim speed (from the literature), performance of surface active behaviors (D. Noren et al., unpublished data) and other behaviors (from the literature) will be used to determine the cumulative energetic costs of different disturbance scenarios. Metabolic costs will be reported in units that can be related to activity budgets and daily prey energy requirements.

WORK COMPLETED

From July 2014 (when FY2014 funds were obtained) through September 2015, all objectives have been initiated and some have been completed, as described below.

Objective 1: The analysis comparing the metabolic costs of distinct sound types (social sounds and clicks) has been completed, and the results have been drafted for publication submission (Holt et al., in prep).

Objective 2: In December 2014 we held a dissection workshop with Dr. Ted Cranford and Dr. Joy Reidenburg (Mount Sinai) where we completed detailed dissections of a harbor porpoise's and a bottlenose dolphin's head to develop sample collection protocols for our muscle histochemistry work as well establish the structures that would be included in the CT scan analysis (Fig. 1). We reviewed over 20 CT scans collected by The California Academy of Sciences through The Marine Mammal Stranding Program and selected the best scans for our investigation. Analysis of CT scan data (see Fig. 2) have been completed, exceeding the number of animals that were originally proposed (total n = 9, 5 species).

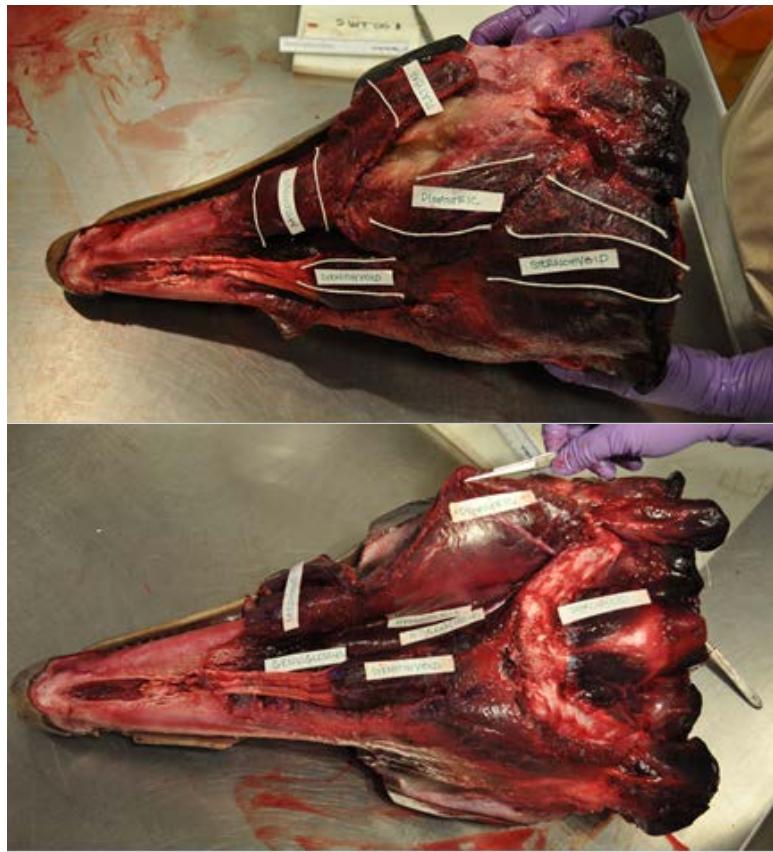


Fig. 1 Examples of detailed dissections completed during the December 2014 dissection workshop. White strings indicate the direction that the muscle fibers are oriented and individual muscles are labeled. Top panel shows the superficial muscles and the bottom panel shows the deep muscles.

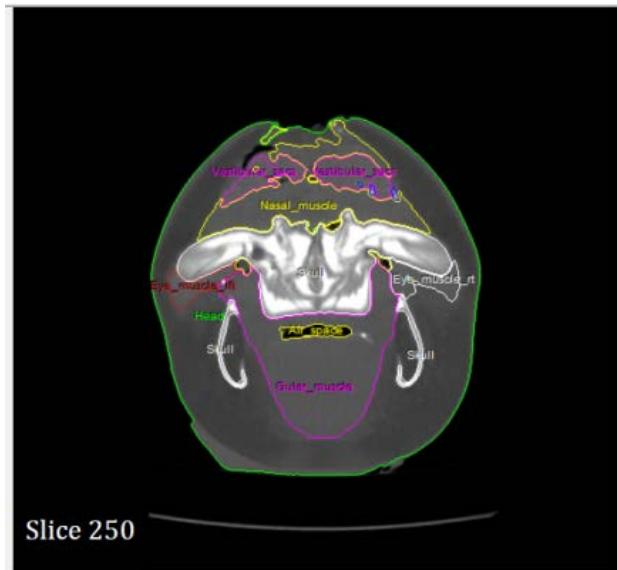


Fig. 2 Example of one CT slice from a harbor porpoise used for the volume of sound production muscle analysis. Gular muscles are outlined in purple, nasal muscles are outlined in yellow. The remaining head structures were grouped and excluded from the total.

Objective 3: We are in the process of using the CT scan results to develop the across-species model of the metabolic cost of sound production.

Objective 4: Muscle samples have been obtained from all target species, except for the beaked whale due to the inavailability of samples (Table 1). Vocal muscles were collected from four bottlenose dolphin specimens, but matched locomotor muscles were not available. A partial set of vocal muscles was collected from a single killer whale, while only a locomotor muscle sample was obtained from a second killer whale. [MB] and AB analyses have been completed for all muscle samples obtained from bottlenose dolphins and killer whales. Analyses of harbor porpoise samples are ongoing and expected to be completed by November 2015. We were able to obtain robust sample sets from four out of five harbor porpoises sampled (Table 1). Unfortunately, one individual we sampled died of severe trauma and thus we were only able to collect a suitable PPS sample. In addition to conducting [MB] and AB analyses, we developed a collaboration with Dr. Jenn Dearolf (Hendrix College) who will be working with us to conduct fiber type analyses on the collected muscle samples.

Table 1 Samples collected and analyzed for determination of the aerobic and anaerobic capacities of odontocete vocal musculature.

Species	Individuals (n)	Muscle Type	Muscle Samples (n)	Samples Analyzed	
				Mb (n)	AB (n)
<i>Tursiops truncatus</i>	4	Right Nasal Musculature (NM)	4	4	4
		Left Nasal Musculature (NM)	4	4	4
		Palatopharyngeal Sphincter (PPS)	4	4	4
		Genioglossus Complex (GGC)	4	4	4
<i>Phocoena phocoena</i>	5	Right Nasal Musculature (NM)	4	2	1
		Left Nasal Musculature (NM)	4	2	2
		Palatopharyngeal Sphincter (PPS)	5	3	3
		Genioglossus Complex (GGC)	4	2	2
		Locomotor Muscle	4	2	2
<i>Orcinus orca</i>	2	Right Nasal Musculature (NM)	1	1	1
		Left Nasal Musculature (NM)	1	1	1
		Locomotor Muscle	2	2	2

Objective 5: Literature reviews on disturbance scenarios and species-specific responses for beaked whales, bottlenose dolphins, harbor porpoise, and killer whales are ongoing. This work relies on data from an earlier study on the energetic cost of performing surface active behaviors in bottlenose dolphins (Noren, Dunkin and Williams, unpublished data). Although most of the data analysis for that

study had been completed before the present ONR award, the analysis required to determine the metabolic cost of individual surface active behavior bouts had not been fully completed. That analysis is in progress. The development of a conceptual model for disturbance scenarios and southern resident killer whales responses was initiated. Variables from other studies that will be incorporated into calculations to estimate the energetic costs of different behavioral responses to disturbance have also been identified for southern resident killer whales.

RESULTS

Preliminary results for objectives 1, 2, and 4 are described below.

Objective 1: Across all click production trials (MBholt report on award no. N0001412IP20072 and Noren et al., in internal review), the average received cumulative energy flux density (cEFD) level of clicks produced per trial was higher than the average cEFD for social sound production trials (difference was +5 dB for Dolphin A and +9 dB for Dolphin B). Although cEFDs of social sounds overlapped with that of clicks, no cEFDs from social sound production trials equaled those from click production trials (see Fig. 3). Thus, the significant linear relationship between metabolic cost and cEFD for social sound production (Fig. 3) was used to predict metabolic cost of social sound production for the same energy content as click production trials for each subject. In the previous analysis, data for each subject were analyzed separately due to the different social sounds produced and the restricted range of vocal effort in Dolphin A (Holt *et al.*, 2015). Data are combined here for simplicity as metabolic costs for equivalent cEFD agree between subjects. Using the predicted equation, the estimated metabolic cost of producing social sounds for a 2 min period at the same average cEFD as the click trials (154 and 159 dB re 1 $\mu\text{Pa}^2\text{s}$) was 1736 and 2404 ml O₂ for Dolphin A and B, respectively (Fig. 3). The metabolic cost of producing clicks is much lower than that of producing social sounds of equal energy (Fig. 3). In fact, the metabolic cost of click production in bottlenose dolphins is considered negligible (MBholt report on award no. N0001412IP20072, Noren et al., in internal review). These empirical results demonstrate that bottlenose dolphins produce clicks at a very small fraction (on the order of ~5%) of the metabolic cost of producing social sounds such as whistles and squawks of equal energy. These findings are consistent with the observation that whistle generation requires considerably higher air pressure within the bony nares than click generation (Cranford *et al.*, 2011).

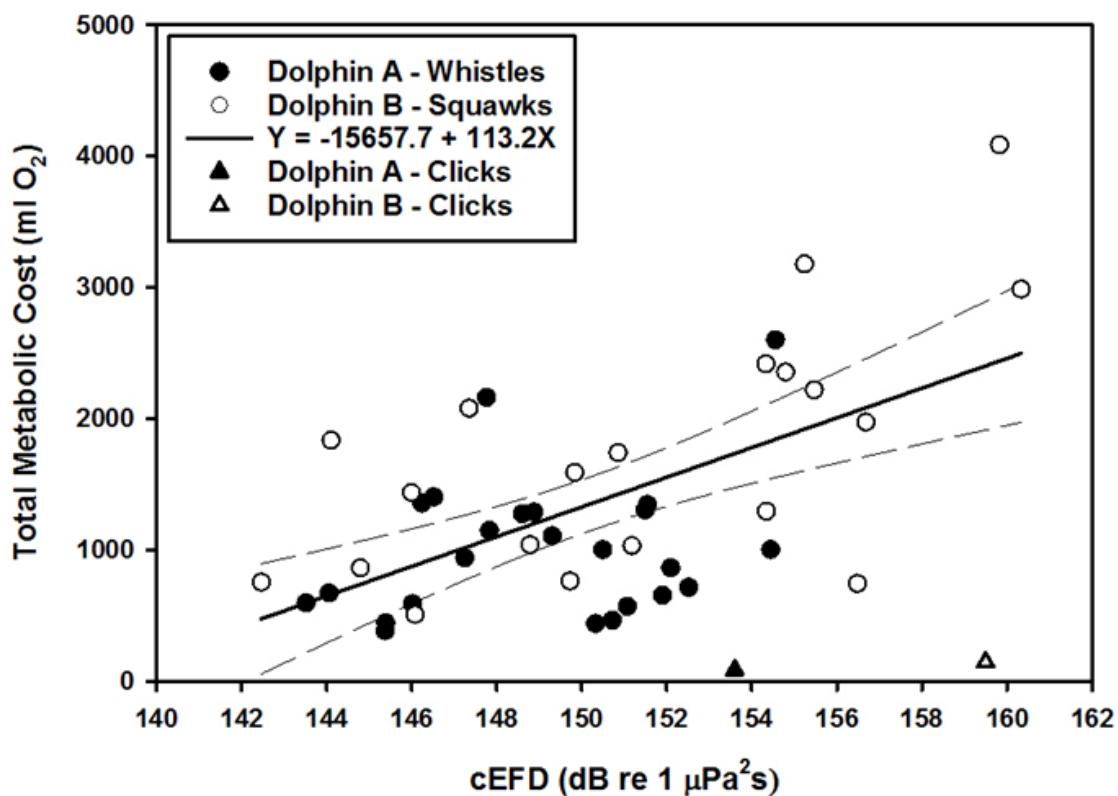


Fig. 3 Total metabolic cost above baseline values plotted as a function of cEFD of sound generation in two bottlenose dolphins. Circles are from individual trials for social sound production (closed = Dolphin A, open = Dolphin B); the solid line and predicted linear regression equation shown in the legend are derived from these individual social sound trials for both subjects combined. Triangles represent the estimated metabolic cost of click production, averaged across all trials for each subject separately (closed = Dolphin A, open = Dolphin B).

Objective 2: Results from the CT scan analyses indicate that the total muscle mass of nasal and gular musculature devoted to sound production as a percent of body mass, is similar across 5 species of odontocetes and comprises approximately $2.2 \pm 0.5\%$ of total body mass (Fig. 4). Furthermore, while the contribution of nasal muscle is relatively constant and low (approximately 0.3% of total body mass), the gular muscle mass contributes the majority of the total mass of sound production muscle (Fig. 5). This result is consistent with the theory that gular muscles provide the power to pressurize the air sacs which is likely the most energetically costly part of sound production.

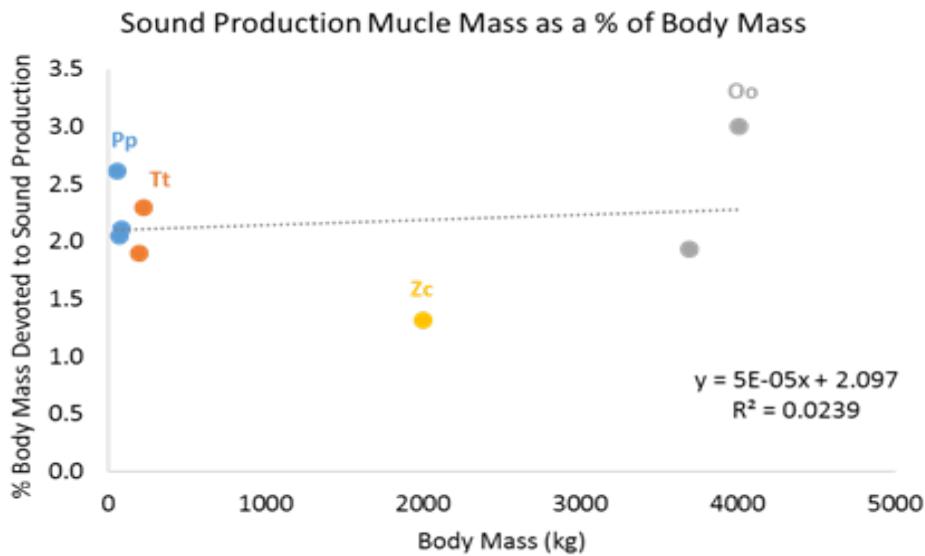


Fig. 4 Sound production muscle mass expressed as a percentage of total body mass plotted against total body mass. Data from harbor porpoise (blue), bottlenose dolphin (orange), beaked whale (*Z. cavirostris*, yellow), and killer whale (gray) are presented. Note that while we have estimated the muscle mass of the sound production muscles for the second beaked whale (*Mesoplodon densirostris*), we are still exploring reliable methods to estimate the mass of this animal based on a known length.

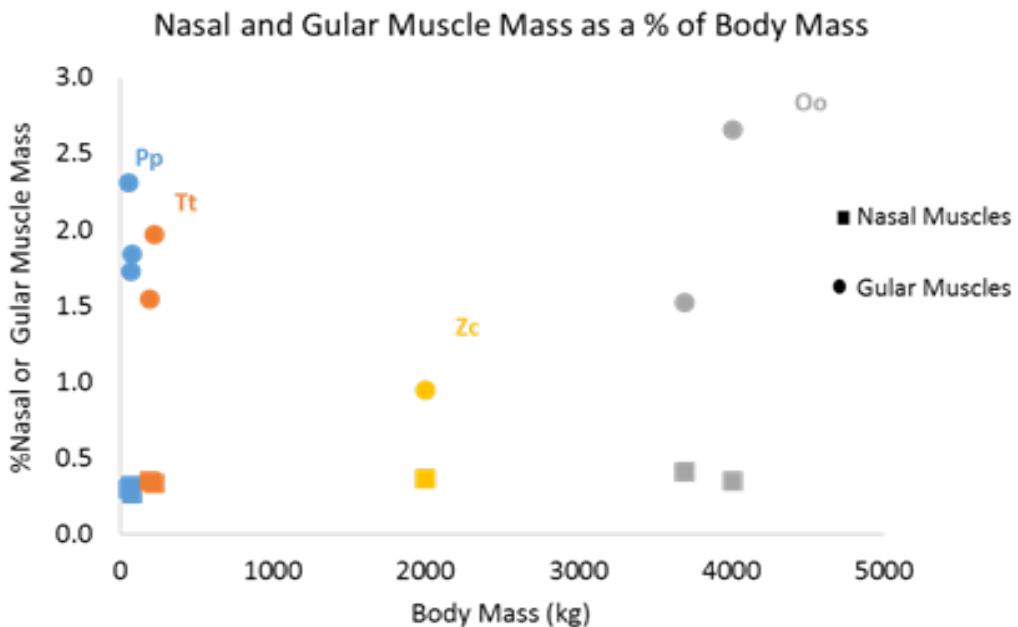


Fig. 5 The mass of nasal and gular muscle mass expressed as a percentage of total body mass plotted against total body mass. Data from harbor porpoise (blue), bottlenose dolphin (orange), beaked whale (*Z. cavirostris*, yellow), and killer whale (gray) are presented. Note that while we have estimated the muscle mass of the sound production muscles for the second beaked whale (*Mesoplodon densirostris*), we are still exploring reliable methods to estimate the mass of this animal based on known length.

Objective 4: Preliminary results suggest that vocal muscles from all three species have reduced aerobic and anaerobic capacities compared to species-specific locomotor muscles. Indeed, [MB] and AB capacities of the vocal muscles measured to date are less than half the species-specific values for locomotor muscles. These data suggest a relatively low energetic cost of sound production compared to locomotion in all three species.

IMPACT/APPLICATIONS

Results of this investigation will provide important quantitative data that can be incorporated into efforts to assess the biological significance of behavioral and acoustic responses to anthropogenic sound exposure including activities with naval relevance. Results of the metabolic cost comparisons between sound types in bottlenose dolphins have specific implications for estimating costs of vocal responses to noise disturbance. Across different sound types, metabolic rates are affected by the acoustic energy of the signals. Results from the efforts to estimate the metabolic cost of sound production across several odontocete species will provide the missing data required to evaluate the energetic impact of disturbance in other species of concern, including killer whales, harbor porpoise, and beaked whales. These results, combined with results from other studies, will be incorporated into models to estimate the energetic impacts of species-specific responses to a range of disturbance scenarios.

RELATED PROJECTS

Dr. Terrie Williams' laboratory is conducting other studies related to this project. The goal of one related study is to assess the physiological costs and potential risks of common responses by cetaceans to anthropogenic noise.

http://www.mmpp.ucsc.edu/Marine_Mammal_Physiology_Project/About.html

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PUBLICATIONS

Holt, M.M., Noren, D.P., Dunkin, R.C., and Williams, T.M. 2015. Vocal performance affects metabolic rate in dolphins: implications for animals communicating in noisy environments. The Journal of Experimental Biology 218, 1647-1654 [published, refereed].